This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 51-F-0016 CUSTOMER NO. 441

FORM APPROVED OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

NATIONAL INSTITUTE OF HEALTH
(b)(2)High, (b)(7)(F)
9000 ROCKVILLE PIKE
BETHESDA, MD 20892
(301) 496-5424

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

See Attached Listing Composite includes: APF,CC, NCI, NEI,NHGRI,NHLBI,NIA,NIAAA,VRC NIAID,NIAID (RML)NIAMS,NICHD,NIDA, NIDCD,NIDCR,NIDDK,NIEHS,NIMH,NINDS,ORS

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs	15	69	150	0	219
5. Cats	9	4	4	0	8
6. Guinea Pigs	103	1130	57	41	1228
7. Hamsters	2696	165	590	20	775
8. Rabbits	107	1335	503	0	1838
9. Non-Human Primates	1053	1585	821	0	2406
10. Sheep	57	15	6	0	21
11. Pigs	55	107	163	0	270
12. Other Farm Animals	--	--	--	---	--
Cattle	0	2	0	0	2
13. Other Animals	--	--	--	---	--
Gerbils	10	0	0	0	0
Ferrets	19	2	0	80	82
Cottons Rats	0	74	0	0	74

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)

(b)(6), (6)(7)(C) ve is true, correct, and complete (7 U.S.C. Section 2143)

SI (b)(6), (6)(7)(C) DATE SIGNED 12/11/07

AP DQUARTERS

(AUG 91)

This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO. 51-F-0016 CUSTOMER NO. 441

FORM APPROVED OMB NO. 0579-0036

CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

NATIONAL INSTITUTE OF HEALTH
(b)(2)High, (b)(7)(F)
9000 ROCKVILLE PIKE
BETHESDA, MD 20892
(301) 496-5424

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
Squirrels	242	328	0	0	328
Wild Mice	207	24	0	0	24
Llama	0	1	0	0	1
Chinchillas	0	54	0	0	54

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)

(b)(6), (6)(7)(C) above is true, correct, and complete (7 U.S.C. Section 2143)

(b)(6), (6)(7)(C)

DATE SIGNED 12/1/09

), w EADQUARTERS

(AUG 91)

EXPLANATION FOR COLUMN E LISTING

This form is intended as an aid to completing the column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: **51-F-0016**
2. Number of animals used under Column E conditions in this study: **17**
3. Species (common name) of animals used in this study: **Hamster**

4. Explain the procedure producing pain/or distress, including reason (s) for species selected.

Leishmanial diseases are major parasitic diseases of man. The stage of the parasite that grows in the vertebrate host and causes disease cannot be generated *in vitro* (cell culture). It can only be obtained from *in vivo* (live animal) sources. In nature, most leishmanial species are maintained within animal reservoirs, usually rodents. The hamster is the only laboratory animal that develops visceral (abdominal) leishmaniasis. There is no way to test the action of vaccines *in vitro* (laboratory conditions). The whole animal is required to study experimental vaccines, protective immune responses and the outcome of infection of vaccinated animals. Information derived from the immune system responses being examined cannot be gathered by using cell culture or computer models. Visceral leishmaniasis in hamsters is manifested by anemia and an enlarged liver. The progression of visceral infection in hamsters is not associated with any overt pathology or changes in behavior until infection is severe, at which time hamsters begin to move slowly and lose their appetite.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

The point of onset of morbidity is variable, but generally occurs in the period 12 - 16 weeks post infection (when parasite inoculum is low and parasites are injected intradermally). Affected hamsters lose significant weight over several weeks and without intervention, become moribund. The only means for pain or distress relief was euthanasia. Analgesics were not used during the two-day period after morbidity was observed because they affect the organs which were evaluated for size, histology, and parasite load as an endpoint to compare vaccinated and non-vaccinated animals.

EXPLANATION FOR COLUMN E LISTING

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: **51-F-0016**
2. Number of animals used under Column E conditions in this study: **3**
3. Species (common name) of animals used in this study: **hamster**
4. Explain the procedure producing pain/or distress, including reason (s) for species selected.

Hamsters infected with *L. donovani* are an established experimental model for visceral leishmaniasis. Animals infected for study served as a source of tissue amastigotes and test candidates for vaccines. The progression of visceral infection in hamsters is not associated with any overt pathology or changes in behavior until the infection is severe. The point of onset of morbidity occurred 6 - 10 weeks period post infection. The disease was progressive with affected hamsters displaying an enlarged liver with anemia. Many became slow moving as time progressed

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Infected hamsters were allowed to progress to fulminating disease to either measure the efficacy of a vaccine or provide enough amastigotes to be harvested.

Analgesics such as NSAIDS and opiates interfere with the inflammatory process (as measured by spleen and liver weights) under study and consequently confound experimental results.
In all cases, hamsters showing signs of illness were euthanized within 2 days of onset.

EXPLANATION FOR COLUMN E LISTING

This form is intended as an aid to completing the column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: **51-F-0016**
2. Number of animals used under Column E conditions in this study: **41**
3. Species (common name) of animals used in this study: **guinea pig**
4. **Explain the procedure producing pain/or distress, including reason (s) for species selected.**

It was essential to study HSV-2 (Herpes Virus-2) candidate vaccines in animal models before clinical testing. No computer simulation or cell culture model can successfully mimic the process. HSV-2 infection in the guinea pig results in painful mucocutaneous lesions similar to those found in man. The vaginal route of infection was used in this study because of our interest in developing vaccines that can protect humans from genital infection of HSV-2.

HSV-2 can also spread to central nervous system resulting in a neuritis and eventually death.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

To determine if the vaccine candidates can prevent or reduce disease caused by the natural virus found in nature (wild-type), guinea pigs were inoculated with vaccine candidates and later challenged with wild-type virus at a dose that causes disease. In some cases we allowed the infection to progress to the point of moribundity so the severity of primary disease and the rate of reactivation of HSV could be measured over time.

The use of analgesics and pain-relieving medicines, (opiates, NSAIDS), would have altered the immune response to the virus and interfere with the interpretation of the experimental results. Opiates have also been shown to interfere with virus reactivation by interacting with receptors on the surface of neurons where the virus maintains in latency.

Column E Explanation Form

1. Registration Number: **51-F-0016**
2. Number of animals used under Column E conditions in this study. **80 .**
3. Species (common name) of animals used in this study. **ferrets**
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

   This project is to develop vaccines to protect humans against respiratory viruses, namely highly pathogenic avian influenza viruses. Viral infection and the induction of an immune response can only be studied in living animals. We are limited in our ability to study these virus infections and vaccines responses in the natural human host or in permissive primate models because of limited availability, limited genetic tools, and ethical considerations. Ferrets are mammalian models to study disease and evaluate potential vaccine candidates. Avian influenza viruses are not uniformly virulent for ferrets. Infection of ferrets with some highly pathogenic avian influenza viruses can result in disease symptoms that can range from very mild disease up to pneumonia and death if unintervened. In this regard, it resembles the rare avian influenza infections reported in humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

   For the attenuation studies, we have conducted studies to evaluate the level of attenuation of live vaccine candidates compared to the wild-type viruses that cause the disease in nature. H5N1 wild-type influenza viruses have been shown to cause severe clinical signs in ferrets (Zitzow et al. 2002). Since the attenuation studies measure the ability of the virus to replicate in the animal, and some influenza virus subtypes cause clinical signs in ferrets, we will not administer antivirals or antipyretics/analgesics to animals that show clinical signs. There are two reasons why nonsteroidal anti-inflammatory drugs (NSAIDs) will not be administered to attenuation-study ferrets that exhibit fever. One reason is that understanding the fever response to these infectious agents is an important endpoint of validating this model and these viruses. Secondly, anti-inflammatory properties of the NSAID may affect the immune response to the viruses, which may affect the course of the disease. However, we have found clinical signs not as severe during the time period of the studies (up to 3 days post-infection).